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Transfer Factor Immunotherapy in Hodgkin's and non-Hodgkin's Lymphoma

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Summary. *Human dialyzable transfer factor was administered in a double-blind fashion to patients with Hodgkin's disease and non-Hodgkin's lymphoma. Two groups were examined; patients with active disease and patients in remission. Parameters of cellular and humoral immunity were studied. The effect of transfer factor on the clinical condition was not evaluated.*

Transfer factor tended to intensify the skin test reactions of patients in remission to several recall antigens, but had no effect on the other parameters or the other patient group.

Introduction

Patients with Hodgkin's disease have been shown to have a broad-based deficiency of cell-mediated immunity (CMI) involving anergy in skin tests to many recall antigens [2, 6, 7, 10], inability to develop delayed hypersensitivity responses to contact sensitizing agents [6, 16], and abnormal in vitro responses to mitogens in lymphocyte culture [5, 17, 25]. Lymphocyte counts tend to be decreased [11, 25]. Humoral immunity, however, seems to be normal, except in patients with very advanced disease [2, 22]. In most cases the immunoglobulins are unchanged after radiotherapy or chemotherapy, but decreased values can sometimes be seen for the amount of IgA and IgM after splenectomy [10, 22]. Impairment of the in vivo and in vitro parameters of CMI in patients with non-Hodgkin's lymphoma has also been described, although these parameters have been less exhaustively analyzed than in Hodgkin's disease [11].

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Very often the blast responses and skin test sensitivity are said to be in correlation with the clinical stage [2, 3, 6, 27]. In some reports, however, this has not been so [30]. The decreased CMI value may persist for more than 2 years after treatment with radio- or chemotherapy [1, 8, 25]. This defect may be in circulating lymphocytes [8].

Cancer immunotherapy has been shown to be of some benefit in many kinds of malignant disease. In lymphomas BCG immunotherapy may have some effect on the prognosis [12, 18, 21]. It has been said to be superior to no treatment after induction of remission [18]. The intrinsic defect in lymphocytes of patients with Hodgkin's disease can be alleviated by Levamisole [1, 21]. Levamisole can also rectify the T cell depletion [21]. Transfer factor preparations may have some effect on skin test values, PHA responses, and T lymphocyte counts in patients with Hodgkin's disease in remission [13, 19, 23]. It is, however, difficult to evaluate the place of immunotherapy in Hodgkin's disease and non-Hodgkin lymphoma, because there have been no strictly randomized double-blind studies.

In this randomized double-blind study we wanted to know the effect of a transfer factor (TF) preparation on the in vivo and in vitro parameters of CMI of patients with Hodgkin's disease and non-Hodgkin's lymphoma.

Materials and Methods

Description of the Patients

A. Patients in Remission. Seventy patients with Hodgkin's disease or non-Hodgkin's lymphoma were examined in the first part of the trial. All these patients had been in remission for more than 2 years and they had previously been treated by radiotherapy and/or chemotherapy.

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B. Patients with Active Disease. Twenty-four consecutive patients with active, previously untreated Hodgkin's disease and 21 similar patients with non-Hodgkin's lymphoma entered the trial. The clinical staging of these patients was performed by lymphography, chest X-ray, bone marrow biopsy, liver, spleen, and skeletal scans, and physical examination. Patients who needed immediate chemo- or radiotherapy were excluded (Table 1).

Measurement of CMI

A. Skin Testing. Tuberculin (PPD, State Bacteriological Laboratory, Copenhagen, Denmark), oidiomycin (OM, dermatophytin 'O', Hollister-Stier Laboratories, Spokane, WA, USA), streptokinase-streptodornase (SK-SD, Varidase, Lederle Laboratories, USA) and parotitis virus (PAR, State Laboratory, Helsinki, Finland) were used as skin test recall antigens. The concentrations of PPD, OM, SK-SD, and PAR were 0.1 TU, 1 TU, 10 TU, and 100 TU; 1:500, 1:50, and 1:5; 1:500 and 1:50; and 1:100 and 1:10, respectively. The skin tests were started with the lowest concentrations and repeated at 3-day intervals with increasing concentrations of antigen until a positive reaction was noticed or the highest concentrations of the test antigen had been used. The test antigens were injected intradermally on the volar aspect of the forearm. Erythema and induration of more than 5 × 5 mm in diameter was regarded as a positive reaction. The results were recorded after 48 h.

The patients were also sensitized with dinitrochlorobenzene (DNCB) according to a chamber method developed by Pirilä (P. Gröhn et al. 1981, unpublished work). DNCB was dissolved in white petrolatum and stored in 10-ml plastic syringes. A concentration of 0.5% (100 μg) DNCB was used as the priming concentration and the patients were tested 2 weeks later with concentrations of 0.03%, 0.1%, and 0.2% DNCB, corresponding to 5 μg, 20 μg, and 50 μg, respectively.

The results of skin tests were calculated according to the following score: tuberculin (PPD): 0.1 TU+, 4; 1 TU+, 3; 10 TU+, 2; 100 TU+, 1; 100 TU−, 0; parotitis (PAR): 1:100+, 2; 1:10+, 1; 1:10−, 0; oidiomycin (OM): 1:500+, 3; 1:50+, 2; 1:5+, 1; 1:5−, 0; streptokinase-streptodornase (SK-SD): 1:5,000+, 2; 1:500+, 1; 1:500−, 0; dinitrochlorobenzene (DNCB): 0.03+, 6; 0.1+, 5; 0.2+, 4; 0.2−, 3.

B. Lymphocyte Cultures. Phytohemagglutinin (PHA, phytohemagglutinin 'P', Difco Laboratories, Detroit, Michigan, USA) and concanavalin A (ConA, Doncanavalin-A, Pharmacia, Uppsala, Sweden) were used as the mitogens in the lymphocyte cultures. The concentrations in the cultures were 1:30, 1:150, and 1:600 for PHA, and 0.5, 5, and 50 μg/ml for ConA. The tests were performed according the method described by Stites et al. [28] and the results were calculated as percentages of normal control values.

Measurements of Humoral Immunity

Classes of immunoglobulins were quantified with the aid of commercial radial immunodiffusion plates of Behringwerke AG.

Radiotherapy

Radiotherapy was started after the immunological tests had been completed and TF administered. A cobolt 60 source or a 6 MeV linear accelerator was used. The mean tumor dose was 40 Gy in 6 weeks with a 2 weeks' split to every treated nodal region.

Statistical Methods

The patient groups were analyzed and compared with each other by using the two-directional analysis of variance method. The statistical significance between the results of the groups was calculated by using the Student's *t*-test for correlated groups [29].

Table 1. Description of the patients with active disease

Patients	Number of patients treated with TF	Number of patients treated with saline
Hodgkin's disease, total subgroups	13	11
Lymphocyte predominance	3	1
Nodular sclerosis	7	7
Mixed cellularity	2	2
Epitheloid type	1	1
Hodgkin's disease		
Stage I + II	11	9
Stage III + IV	2	2
Non-Hodgkin's lymphoma, total	10	11
Stage I + II	4	6
Stage III + IV	6	5
Female	10	7
Male	13	15
Mean age (yrs, range)	43.4 (18–71)	36.4 (15–63)

Preparation of TF

TF was prepared from buffy coat cells from healthy blood donors (Finnish Red Cross Blood Transfusion Service, Helsinki, Finland). Though the immune status of the donors was not tested, it is known that only 10% of the population in Finland has a negative skin reaction to 10 TU PPD [24]. The buffy coats were pooled, mixed with half their volume of 6% dextran (Macrodex, Leiras Pharmaceuticals, Turku, Finland), and kept at 37° C for 60–90 min. The leukocyte-rich supernatant was then collected and centrifuged at 250 *g* for 20 min; the cells were then washed three times with cold phosphate-buffered saline, mixed, and counted in a hemocytometer. The yield per unit (450 ml) of blood was $8-10 \times 10^8$ lymphocytes, corresponding to 3.3×10^9 leukocytes. The cells were disrupted by freezing and thawing ten times (−20° C, 37° C), and ultrafiltered through a dialysis membrane (Visking tubing, Union Carbide Co., Chicago, Ill., USA) at a pressure of one atmosphere. The ultrafiltrate was concentrated by lyophilization, sterilized by Millipore filtration (0.22 μm), and divided into doses of 1 ml, corresponding to 3 U blood (1×10^{10} leukocytes). The TF tubes were stored at −20° C. This leukocyte ultrafiltrate has been shown to contain a nonspecifically active TF compound [9].

General Plan of the Study

Seventy patients with Hodgkin's disease and non-Hodgkin's lymphoma in remission were randomly allocated to receive three injections of TF or saline at weekly intervals. Each injection of TF corresponded to the yield from 3 U blood (10^{10} leukocytes). The patients' immune function was tested before any treatment, after three injections of TF/NaCl, and immediately after completion of radiotherapy.

Patients with active disease were randomized to receive TF or saline in a double-blind fashion. The three TF and saline injections were given at weekly intervals. Thereafter, the patients received radiotherapy to supra- or subdiaphragmatic regions according to the stage and localization of the disease. The test measuring CMI were performed before any treatment, after the TF or saline injections, and after the radiotherapy. The effect of the immunotherapy on the clinical condition of the patients was not evaluated.

Results

The effect of treatment with TF or saline on the skin test values of lymphoma patients in remission.

Forty patients with Hodgkin's disease and 30 with non-Hodgkin's lymphoma were randomized in the study. Three patients in the former and seven in the latter group refused the trial after the randomization because of weak personal motivation. In the Hodgkin's disease group 18 patients received TF and 19 saline, and in the Hodgkin's lymphoma group nine patients received TF and 14 saline.

The skin test values of the patients receiving TF increased significantly with every antigen (Table 2). This was also seen with two antigens in the control group. Increased reactivity to the antigens was found in TF-/NaCl-treated groups as follows: PPD 15/27 (TF), 14/33 (NaCl); PAR 11/27 (TF), 10/33 (NaCl); OM 14/27 (TF), 17/33 (NaCl) and SK-SD 15/27 (TF), 8/33 (NaCl). Though the difference between the groups was not statistically significant the values tended to improve more in the TF-treated group.

Skin Tests of Patients with Active Disease

In this material the skin test reactivity was almost equal in patients with limited disease (stage I–II), patients with extensive disease (stage III–IV), and patients with B symptoms.

In each group decreased skin test values were seen in some patients, but this was not dependent on the stage of the disease. Compared with values from a normal healthy population [9] the values were markedly lower in all groups (Table 3).

Table 2. The effect of treatment with TF or saline on the skin test values of lymphoma patients in remission

Patients	PPD			PAR			OM			SK-SD		
	Before	After	*P* value	Before	After	*P* value	Before	After	*P* value	Before	After	*P* value
Hodgkin's disease												
TF-group (18)	2.6	3.2	0.001	0.7	1.0	0.01	1.7	2.2	0.01	1.1	1.8	0.001
Saline group (19)	2.6	2.9	0.02	1.0	1.1	NS	1.0	1.1	NS	1.5	1.8	0.01
Non-Hodgkin's lymphoma												
TF-group (9)	1.8	2.3	0.05	0.7	1.2	0.05	1.4	2.2	0.05	1.1	1.6	0.05
Saline group (14)	2.6	2.9	NS	0.9	1.5	0.01	1.6	1.9	NS	1.3	1.3	NS

Abbreviations: PPD, tuberculin; PAR, Parotitis; OM, oidiomycin; SK-SD, streptokinase-streptodornase; NS, not significant
Grading of the skin tests: PPD, 0.1 TU+ = 4, 1 TU+ = 3, 10 TU+ = 2, 100 TU+ = 1, 100 TU− = 0; PAR, 1:100 = 2, 1:10+ = 1, 1:10− = 0; OM, 1:500+ = 3, 1:50+ = 2, 1:5+ = 1, 1:5− = 0; SK-SD, 1:5000+ = 2, 1:500+ = 1, 1:500− = 0

Effect of TF on CMI of Patients with Active Disease

A. Effect of TF on Skin Test Values. In all groups the reactivity in similarly repeated skin tests seemed to increase in the case of each recall antigen as well as with DNCB (Table 4). The increase was almost equal in patients receiving TF and those receiving saline. The results were analyzed further, as it was suspected that the effect of TF might be seen only in some subgroups of patients. The skin test results were examined according to histology (Hodgkin's disease, non-Hodgkin's lymphoma) and to the stage of the disease. It could be seen that in every subgroup the skin test reactions became stronger almost equally, suggesting that the increased reactivity was due to the repetition of the skin tests. Because it is possible that TF cannot increase the normal CMI reactions, but only depressed reactions, those patients which were anergic to different recall antigens and DNCB were examined separately. Again, no effect due to TF could be demonstrated (Table 5). The only finding that possibly could have been due to TF was the increase in DNCB reactivity, but even this was not statistically significant.

Immediately after the completion of the radiotherapy the skin tests with all recall antigens and DNCB showed decreased values. The change seemed clear but was not statistically significant. This could not be prevented by TF treatment and the only significant decrease (SK-SD) was seen in the group treated with TF (Table 4).

B. Effect of TF on Lymphocyte Cultures. The blast transformation tests were performed in nine patients treated with TF and eight with saline. Initially the mean responses to PHA and ConA were decreased, varying from 28%–72% of healthy control values with different mitogen concentrations. No change was seen in the groups treated with TF or saline.

C. Effect of TF on the Absolute Numbers of Granulocytes, Lymphocytes, and Monocytes. The absolute numbers of granulocytes, lymphocytes, and monocytes remained unchanged during the administration of TF or saline. The absolute numbers of granulo-

Table 3. The skin test values in patients with local (stage I + II) and extended (stage III + IV) diseases and with B symptoms before any treatment

Patient group	Number of patients	Antigens used				
		PPD	PAR	OM	SK-SD	DNCB
Healthy Finnish adult controls	47	3.1		2.1		
Local disease	30	2.6	1.1	1.3	1.3	4.5
Extensive disease	15	2.8	1.1	1.5	1.1	4.3
B symptoms	8	2.8	1.1	1.5	1.2	4.5
Hodgkin's disease	23	2.6	1.2	1.4	1.2	4.4
Non-Hodgkin's lymphoma	21	2.6	1.3	1.5	1.4	4.3

Abbreviations: for antigens used and the grading of the skin test values see Table 2

Table 4. Skin test values of all patients during treatment

Antigen	Treatment group	Before treatment	After treatment	*P* value	After radiotherapy	*P* value
PPD	TF	2.8	3.1	NS	2.2	NS
	Saline	2.4	3.0	NS	2.0	NS
PAR	TF	1.2	1.3	NS	0.9	NS
	Saline	1.0	1.1	NS	0.8	NS
OM	TF	1.5	1.8	NS	0.7	NS
	Saline	1.3	1.6	NS	0.9	NS
SK-SD	TF	1.3	1.5	NS	0.7	0.01
	Saline	1.3	1.6		1.0	NS
DNCB	TF	4.5	5.1	NS	4.6	NS
	Saline	4.2	4.7	NS	3.8	NS

Abbreviations: for the antigens used and the grading of the skin test values see Table 2

Table 5. The effect of TF and saline on the decreased skin test values of all patients with active disease

Antigen	Treatment group	Number of patients	Before treatment	After treatment	*P* value	After radiotherapy	*P* value
PPD	TF	8	1.7	2.5	NS	0.6	NS
	Saline	10	1.6	2.4	NS	1.7	NS
PAR	TF	13	0.7	1.2	NS	0.4	NS
	Saline	16	0.7	1.1	NS	0.8	NS
OM	TF	12	0.6	1.1	NS	0.4	NS
	Saline	12	0.7	1.0	NS	0.5	NS
SK-SD	TF	14	0.9	1.4	NS	0.4	NS
	Saline	13	0.7	1.2	NS	0.7	NS
DNCB	TF	6	3.4	4.0	NS	3.0	NS
	Saline	9	3.3	3.5	NS	3.3	NS

Abbreviations: for the antigens used and the grading of the skin test values see Table 2

Table 6. Absolute numbers of granulocytes, monocytes, and lymphocytes in the peripheral blood of patients with active disease in the course of treatment

Cell type	Treatment group	Before treatment	After treatment	*P* value	After radiotherapy	*P* value
Granulocytes	TF	9.2×10^9/l	9.0×10^9/l	NS	5.7×10^9/l	0.01
	Saline	8.7×10^9/l	9.4×10^9/l	NS	4.5×10^9/l	0.01
Monocytes	TF	0.5×10^9/l	0.6×10^9/l	NS	0.7×10^9/l	NS
	Saline	0.7×10^9/l	0.6×10^9/l	NS	0.6×10^9/l	NS
Lymphocytes	TF	1.9×10^9/l	2.0×10^9/l	NS	0.8×10^9/l	0.05
	Saline	2.2×10^9/l	2.6×10^9/l	NS	0.4×10^9/l	0.01

Abbreviations: NS = not significant

cytes and lymphocytes tended to decrease less in patients receiving TF during the radiotherapy, but the difference was not statistically significant (Table 6).

Effect of TF on Humoral Immunity

Humoral immunity remained unchanged throughout the study in both treatment groups. IgA, IgG, and IgM concentrations were within normal limits and were not affected by TF or radiotherapy.

Discussion

Decreased CMI is often associated with malignant lymphoma, and especially with Hodgkin's disease. There are opposing opinions about the correlation of normal and depressed CMI with clinical stage of the disease [2, 3, 30]. Radio- and chemotherapy have both been reported to depress CMI further [8, 15].

TF from healthy donors has been suggested to have a positive effect on CMI with Hodgkin's disease [13, 19, 23]. Immunotherapy with a nonspecifically acting immunostimulant like BCG has also been reported to increase symptom-free survival [18, 26]. However, the reports are preliminary, and strictly randomized controlled studies have not been performed. Under these circumstances it seemed to us that an attempt at improving delayed hypersensitivity reactions with TF would be worthwhile. The TF preparation used here has been shown to be active in our previous studies concerning various kinds of patients with chronic infections. The donors of TF were drawn from a healthy Finnish population. There is little information on the dose of TF that should be used in clinical studies. The dose used here represents the yield from 3 U blood. In all, the patients thus received dialysate from 3×10^{10} leukocytes. In infections less than 1 U is reported to be effective [14]. In the treatment of malignancies the dose of TF usually used is equivalent to $1 \times 10^8 - 1 \times 10^9$ lymphocytes [4, 20] and improvement in delayed hypersensitivity in Hodgkin's disease has been reported

with TF obtained from $3-4.9 \times 10^9$ leukocytes [13]. Almost all Finnish people have been vaccinated with BCG in the first year of life. Therefore, most people are positive to 0.1 or 1 TU PPD. The normal skin test reactivity to oidiomycin and PPD is known from our previous studies [9].

In the first part of the study with patients in remission the skin test values to PPD and OM were subnormal before the treatment with TF compared with a healthy Finnish adult population. In the group receiving TF the responses to different antigens increased significantly with every antigen used. The difference from a control group was not significant. As our TF preparation was obtained from normal healthy donors, the effect seen may be due to either specific or nonspecific stimulation.

The patients with active disease also showed impaired responses to skin tests and impaired blast transformation responses, but the quantities of the measured antibody classes were within normal limits. The CMI seemed to be decreased equally in Hodgkin's disease and non-Hodgkin's lymphoma. We could not find any correlation between the impairment of CMI and the clinical stage of the disease, which is in accordance with the findings of other authors [30]. The absence of this correlation may have been determined partly by the exclusion from the trial of those patients who needed urgent therapy.

The skin test reactions became stronger to almost the same degree after administration of TF and of saline, showing that TF cannot overcome the depressed CMI associated with active lymphoma. No change was observed in blast transformation reactions to PHA and ConA during the treatment. After radiotherapy the skin test values decreased in both treatment groups, and this could not be prevented by TF treatment.

The absolute numbers of lymphocytes, granulocytes, and monocytes were normal before any treatment in patients with active disease. No change was noticed during TF treatment. After the radiotherapy the lymphocyte and granulocyte counts were significantly lower than the pretreatment values. The lymphocyte and granulocyte counts, however, tended to decrease less in patients treated with TF than in the control group, although the difference did not reach statistical significance. No change was observed in immunoglobulins during the radiotherapy. The clinical effect of TF was not evaluated. According to our results TF may have a CMI-reconstituting effect in lymphoma patients in remission but not in patients with active disease. This finding indicates the need for a study of the effect of TF on duration of remission and survival in lymphoma patients.

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